## REMARKS/ARGUMENTS

Reconsideration of this application is requested. Claims 1-25 are in the case.

## I. THE 35 U.S.C. §112, SECOND PARAGRAPH, REJECTION

Claims 26 and 27 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. In response, those two claims have been canceled without prejudice. Withdrawal of this rejection is requested.

## II. THE PRIOR ART REJECTIONS

Claims 1, 2, 4, 13, 18, 21 and 23-27 stand rejected under 35 USC 102(b) as allegedly anticipated by or, in the alternative under 35 U.S.C. §103(a) as allegedly unpatentable over Penkowa (2002; Journal of Comparative Neurology 444(2):174-189) as evidenced by Sigma M9542 and Garrett (2000; The Prostate 43:125-135). Claims 1-2, 4,13,17-18, 21, and 23-27 stand rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Giralt (2002; Experimental Neurology 173:114-128, available online 25 February, 2002). Claims 18, 20-25, and 27 stand rejected under 35 U.S.C. 102(b) as allegedly anticipated by FR 2813529 (hereinafter '529 publication), cited on the IDS filed 13 December 2004. Claims 18-25 and 27 stand rejected under 35 U.S.C. 103(a) as allegedly unpatentable over FR 2813529. Claims 1-2, 4, 6-13, 18, 21, and 23-27 stand rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Penkowa (2002; Journal of Comparative Neurology 444(2):174-1 89). Claims 1-13 and 18-27 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Penkowa (2002; Journal of Comparative Neurology

444(2):174-189) in view of FR 2813529, cited on IDS filed 13 December 2004. Claims 1-2, 4, 6-13, 15, 18, 21, and 23-27 stand rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Penkowa (2002; Journal of Comparative Neurology 444(2):174-1 89) in view of Asanuma (2002; Neuroscience Letters 327:61-65; available online 21 April 2002). Claims 1-2, 4, 6-14, 16, 18, 21, and 23-27 stand rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Penkowa (2002; Journal of Comparative Neurology 444(2):174-189) in view of Walsh (US Patent Application Publication 2002/0155170, published 24 October 2002, filed 30 November 2001, claiming benefit of a provisional application filed 30 November 2000). The rejections are respectfully traversed.

As claimed, the invention provides a method of stimulating neuronal regenerative growth or repair. The method comprises exposing a target neuron or neuronal area to a solution of the metallothionein isoform MT-IIA. The focus of the claimed invention is on the administration of metallothionein to promote the **regenerative growth** of neurons following injury. In light of the extensive experimental data undertaken and determined by the applicants and clearly described in the present, the ability of administered metallothionein to promote regenerative growth is a result of **direct action** of metallothionein on the neurons themselves. This direct action without the agency of other cell types or physiological processes is novel and innovative. In particular, this is not described or suggested by the citations relied on in the outstanding Action.

Referring to the prior art rejections over Penkowa and Giralt, Penkowa et al. describes use of metallothionein to **protect** a central nervous system during neuroglial degeneration induced by 6-aminonictinamide. Penkowa examines changes in

numerous cell types but not in neurons. The materials and methods section of Penkowa details antisera used, but none are directed to identification of neurons, and are in fact specific for non-neuronal type cells. Regenerative growth of neurons is not in any way discussed or suggested and no techniques are used which would allow for such detection of regenerative growth neurons. There are no statements or indications whatsoever to imply that metallothionein has an effect on neuronal regeneration.

Giralt et al describes the use of metallothionein to **protect** the central nervous system after a focal brain injury. Giralt et al examines the effect of metallothionein administration or over expression on cells of the immune system, macrophages, microglia, astrocytes, but **not** the effect of metallothionein administration on neurons. Giralt is not concerned with investigating the effect of metallothionein administration on neurons, as no supporting data and disclosure of such steps are found in this publication. The regenerative growth of neurons is not in any way examined or investigated by Giralt and, in fact, neurons are not examined or investigated at all. Giralt contains no indication or suggestion that metallothionein has an effect on neuronal regeneration.

Both Penkowa and Giralt show that metallothionein administered outside of the brain leads to protection of neuronal tissues following traumatic or chemical injury. The authors of both references conclude that this was a result, at least in part, by altering the peripheral (that is the outside of the brain) immune system of animals.

Turning to the Action, the first paragraph on page 4 states that "while the reference (Penkowa) did not explicitly measure "stimulating neuronal growth or repair" as recited in claim 1, it is **reasonable that these in fact did occur,** as Penkowa

performed all the active steps **recited in the method** (i.e. by administering the MT-2 to a subject)." (Emphasis added). The Action goes on to say:

> "Furthermore it is noted that those phenomena measured by Penkowa are consistent with increased health of brain tissue, much as simulating repairs as associated with increased health of the tissue".

The implication from the above is that Penkowa describes the same active steps as the present invention and thus it would be expected that the same outcome is obtained. In response, the experimental model used by Penkowa could not have any way been used and applied to demonstrate the ability of metallothionein to promote regenerative neuronal growth by a **direct action of metallothionein on neurons**, as clearly described and demonstrated in the present application. The following reasons apply:

Looking more closely at the method described by Penkowa, metallothionein was administered intraperitoneally, that is, outside of the brain, and in such a way that it became present in high levels in the blood. Indeed, the authors conclude that the elevated levels of **blood** metallothionein affected the peripheral immune system, and that that, in large part, was the cause of their observed results (protection of brain tissue). In fact, the authors present no evidence that metallothionein can directly affect neurons, nor do they state this anywhere in their work, as a conclusion or as a speculation. Thus, the authors point to an indirect action of metallothionein on the central nervous system.

Penkowa surmise that metallothionein, after intraperitoneal administration, entered the brain. However, **no** evidence is shown for this. It is merely a statement

without proof. Even if metallothionein did enter the brain, there is no evidence (or likelihood) that it reached the concentrations shown by the present inventors to be important in exerting a direct effect on neuronal regeneration.

The present inventors, on the other hand, developed cultured neuron models in which the metallothionein was applied to neurons alone, without the agency of other cells or tissues (i.e. directly to neurons). Furthermore, in the inventors' *in vivo* injury model, they injected metallothionein directly into the brain, so that injured neurons were directly exposed to a concentration of metallothionein that were demonstrated to be bioactive in tissue culture. The present inventors were able therefore to conclude that metallothionein was able to act on neurons directly, and to quantitate how much metallothionein was required to do this.

The Action further suggests that the increase in the health of the brain tissue must have produced "repaired" neurons. This second issue involves the question of whether a treatment which produced an "increase health" of brain tissue, reasonably leads one of ordinary skill to assume neurons will be undergoing regenerative growth. In this regard, the first question to be considered is whether the **protection** of neuronal tissue or cells is the same thing as promoting **regenerative growth** of neurons after injury. A second question is whether it would have been obvious to a person of ordinary skill in the field, knowing that metallothionein is protective, that it would also promote regenerative growth. It is believed that the ability to confer protection and regeneration are distinct and fundamentally different properties driven by different mechanisms and that the ability to confer one property does not allow the prediction of the other property.

A neuroprotectant would not be expected to promote regenerative growth. Hence, knowledge that metallothionein can confer neuroprotection (increased "health" of a tissue, in the language of Penkowa et al) **cannot** lead to the reasonable assumption that metallothionein promotes **regenerative** growth of neurons. The applicants' invention as claimed is specifically the discovery that metallothionein directly promotes **regenerative** growth of neurons by a mechanism involving direct interaction between metallothionein and the neuron.

**Neuroprotection vs Regeneration**

It is well established that neuroprotection and regeneration are distinct and fundamentally different processes, which are part of a sequence of discrete events that must occur if CNS injury is to be treated. One simple way of distinguishing between neuroprotection and regeneration is that they occur at different times after the initial injury. Death of neurons (i.e., the process which is blocked by neuroprotectants) occurs 1-2 days after injury. Regeneration of neurons, however, does not occur until 4-7 days after injury (see the schematic below).

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| Injury | → | Protection and survival of neurons | → Regenerative growth | → Reconnection of neural network |

Neuroprotection ("protection") refers to the ability of compounds or treatments to promote the survival of neural tissue. It sometimes refers to the "sparing" of tissue at the gross level, and sometimes to the survival of specific cells or populations of cells. Neuroprotectants might, for example, have the ability to scavenge free radicals, or block the influx of calcium or other ions into neurons, which would otherwise lead to death of

the cell. Some neuroprotectants activate pathways within neurons that increase the level of endogenous molecules with these attributes, such as glutathione. At a mechanistic level, neuroprotectants ultimately block activation of specific intracellular pathways in neurons which lead to apoptosis or necrosis, processes which result in death of the cell.

Thus, neuroprotection is about survival of cells, or reducing **damage** to cells, including neurons, in the face of injurious conditions, with no imputation about the regenerative capacity of these cells, other than the obvious condition that a neuron must first be alive if it is to regenerate. The action of a neuroprotective agent or treatment can be determined experimentally in several ways, for example, by i) comparing the survival of cells in animals or cultures treated, or not treated, with the putative neuroprotectant, or, by measuring activity of components of apoptotic or necrotic pathways within cells including neurons or ii) indirectly by observing indicators of damage such as oxidation of proteins or other cellular molecules. Penkowa et al, and Giralt et al, use each of these approaches to reach their conclusions.

Regeneration ("regenerative growth of neurons") on the other hand is a fundamentally different process. Regeneration is often triggered by external factors such as growth factors binding to membrane bound receptors on the neuron, intracellular pathways (which are separate to those promoting cell death) are triggered leading to qualitative and quantitative changes in gene expression, producing the regulatory and structural molecules needed for axonal and dendritic growth. Regenerative growth of neurons can be identified visually at later stages of the process: the neuron extends processes, termed "neurites", one of which may develop into an

axon. Neurites are tipped with "growth cones", and are associated with elevated levels of proteins which are not usually observed in neurons not in a growth state. Examples of such proteins included phosphorylated -neuro filaments detected by the antibody SMI312, GAP43 and so on. Thus, regenerative growth of neurons can be determined experimentally by a variety of techniques, including i) direct observation of neurite outgrowth (for example, in culture models) or immunohistochemistry for growth associated proteins or ii) "back labelling" neurons for example by use of the dye DiI which is particularly useful for confirming regenerative growth in animal models. A neuron undergoing regenerative growth can therefore be distinguished from one which has merely "survived".

It is important to note that it is possible, in rare cases, for an agent to have separate neuroprotective and neuroregenerative actions. However, it is not possible to predict or conclude that an agent will promote neuroregenerative growth, based on prior knowledge of its neuroprotective capacity. In fact, there are many neuroprotective agents which do not have neuroregenerative properties (i.e.: glutamate receptor antagonists such as MK-801 or memantine provide some neuroprotection following injury, but no one would suggest that they are also neuroregenerative agents).

Metallothionein appears to possess both neuroprotective and neuroregenerative properties. The papers referred-to by the examiner (Penkowa et al.; Giralt et al.) explore only the neuroprotective capacity of metallothionein and, indeed, they are concerned with neuroprotection at the entire brain tissue level (the term neuroprotection is used loosely to refer to all brain tissue), not with the survival of neurons *per se*. Their very limited use of the term "regenerative growth" refers explicitly to development of an

astrocytic scar (see below), a process involving astrocytes and fibroblasts. On the other hand, the present application is concerned with the ability of metallothionein to directly promote the regenerative growth of neurons, in the aftermath of injury or damage.

The present application shows clearly that (i) in cell culture models, metallothionein promotes neuronal regenerative growth, does not require the agency of non-neuronal cell types, and it appears to act directly on neurons and ii) analogous regenerative growth, as characterised by staining for GAP43 and SMI312, and by DiI back labelling, appears to occur in animal models.

Based on the above, it is clear that invention as claimed is not anticipated or rendered obvious by Penkowa or Giralt. Withdrawal of the anticipation/obviousness rejections based on those two references is requested, as well as withdrawal of the obviousness rejections based on Penkowa and Giralt taken alone or in conjunction with secondary art (FR 2813529, Asanuma, and Walsh).

With regard to the rejections of claims 18, 20-25, and 27 under 35 U.S.C. 102(b) as allegedly anticipated by FR 2813529 (hereinafter '529 publication), and claims 18-25 and 27 under 35 U.S.C. 103(a) as allegedly unpatentable over FR 2813529, it is believed that those rejections should be withdrawn since the cited French patent neither anticipates nor renders unpatentable the subject matter of the therapeutic composition claims. Such action is respectfully requested.

## III. CLAIM AMENDMENTS

Claim 1 has been amended to introduce the word "regenerative" to qualify the neuronal growth. Basis appears at page 5, line 5. Minor revisions have been made elsewhere to improve the form of the claims. No new matter is entered.

Favorable action is awaited.

Respectfully submitted,

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